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Linking Environmental Particulate Matter with Genetic Alterations

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In their article, Tarantini et al. (2009) focused on the basic question of mutual relationships between environmental and genetic factors. From a more general point of view, this also involves the question concerning the relative role of "intrinsic toxicity" of xenobiotics and individual susceptibility or host response. In particular, data from epidemiologic and *in vitro* studies must cope with pathologic evidence for pathologists, as well as cause-and effect-relationships for pathophysiologists. In between are inferences and extrapolations on the basis of plausibility.

Tarantini et al. (2009) showed, for the first time in humans, that reactive oxygen species (ROS)—which are considered one of the main cellular stressors generated by PM exposure—may produce genomic hypomethylation and increased expression and activity of inducible nitric oxide synthase (*iNOS*) not only *in vitro*, but in humans exposed to particulate matter (PM). Although this finding is expected, it is a step forward, based on DNA adduct generation produced by polycyclic aromatic hydrocarbons (PAHs) and other PM components, namely transition metals. Alterations of DNA methylation of the promoter is a common finding in environmental-related chronic or cancerous diseases.

Alterations in DNA methylation and iNOS methylation, as observed by Tarantini et al. (2009) in association with exposure to PM < 10 μm in aerodynamic diameter, may represent an initial step in reproducing decreases in global DNA methylation content that are eventually observed in cardiovascular diseases and cancer.

However, pathologists and pathophysiologists are required to interpret more correctly what this means. Subjects with inherited multitumoral syndromes have a germline mutation, usually a point mutation present in all cells of the body, which determines the occurrence of multiple tumors in the same individual. The occurrence of DNA adducts or mutations in some cells or tissues due to exposure to PAHs or diesel exhaust does not necessarily induce clinically evident outcomes in the future, because each individual is endowed with a wide variety of natural defenses and repair mechanisms that usually overcome every type of DNA damage. Therefore, the first inference to avoid is that DNA adduct formation or alterations in the promoter methylation of a gene causes cancer in the absence of inherited or acquired predisposition (i.e., a point germline mutation of a tumor suppressor gene or an acquired sporadic mutation). In addition, even in patients with inherited multitumoral syndromes (i.e., in subjects with germline mutations of suppressor genes), tumor occurrence (type and severity) is also greatly influenced by epigenetic factors, environmental stimuli, or even long-distance catastrophes. We previously demonstrated an increased incidence of papillary thyroid carcinoma in three members of the same familial adenomatous polyposis (FAP) family (i.e., a kindred having a 1061 *APC* gene mutation). This mutation is responsible for FAP, in part as a side effect (long-term–long-distance) after the Chernobyl disaster, thus suggesting a wider than expected impact of environmental disasters in predisposed subjects (Cetta et al. 1997, 2000).

Analogously, chronic exposure to toxic or carcinogenic environmental substances does not elicit the same results in all individuals. The final clinical outcome (cancer, asthma, pulmonary fibrosis, atherosclerosis, or coronary diseases) seems to be less dependent on the toxic potency of the pollutant or of the exposure dose and more on the individual susceptibility of the host (Cetta 2009a, 2009b). This approach should facilitate a better understanding of the < 5% incidence of mesotheliomas in subjects with the same chronic exposure to asbestos, or the absence of health effects in husbands with chronic occupational exposure to asbestos but the occurrence of mesotheliomas in wives with minor indirect exposure from their husbands (Cetta F, unpublished data).

However, acute and chronic inflammation is the first pathological step. The final clinical outcome (e.g. cancer) does not depend on the first DNA adduct formed or a genetic mutation produced by xenobiotics. A long, automaintaining process will start, such as in liver cirrhosis, leading to cancer or chronic alterations as the final result of the interactions between host and the pathogenic agent. This process is greatly influenced by individual susceptibility or resistance. In PM-related diseases, a major role—in addition to the intrinsic toxicity of the xenobiotic—is played by individual host susceptibility and reactivity, similar to what occurs in autoimmune or autoinflammatory diseases.

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Environmental Particulate Matter and Genetic Alterations: Tarantini et al. Respond

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We thank Cetta et al. for the interest they express in our recent article (Tarantini et al. 2009). The basis for our hypothesis that foundry workers exposed to air particles might have hypomethylation of DNA repetitive element was existing evidence demonstrating that inhaled airborne particles induce oxidative stress and *in vitro* studies indicating that oxidative stress might generate hypomethylation throughout the genome. We are glad to see that Cetta et al. also find such hypothesis well grounded in previous existing work. Our investigation showed for the first time that airborne particles induce hypomethylation in repetitive sequences that are widely represented across the human genome, and indicated that hypomethylation of the inducible nitric oxide synthase (*iNOS*) gene is one potential mechanism contributing to particle-induced oxidative stress and inflammation. In addition, we showed that

such processes can be detected in a DNA source, such as peripheral blood leukocytes, which is easily obtainable from human subjects. Our findings might be extended to ambient air pollution exposures, as suggested by our related investigations demonstrating repetitive element hypomethylation, as well as other gene-specific modifications, in blood DNA from individuals exposed to airborne benzene (Bollati et al. 2007) or to ambient particulate matter (Baccarelli et al. 2009).

In their letter, Cetta et al. rightly emphasize the possible roles of personal genetic features in determining which individuals will develop adverse health outcomes in response to air particle exposure. This is also confirmed by several other investigations that evaluated different health-related end points, including our previous work demonstrating genetic polymorphisms in pathways related to oxidative stress responses (Chahine et al. 2007) and our results on methyl nutrient metabolism (Baccarelli et al. 2008), both of which augment the negative effects of ambient particulate matter on cardiac autonomic function. In comparison with genetic variations, DNA methylation and other epigenetic modifications are of particular interest with respect to air particle effects, because—as demonstrated by animal models of environmental epigenetic toxicity —they are reversible and may be restored to their original state by dietary or pharmacologic interventions (Baccarelli and Bollati 2009; Dolinoy and Jirtle 2008; Jirtle and Skinner 2007).

We agree that the investigation of mechanisms closer to the final health outcomes in the chain of events started and/or maintained by air pollution exposures is as important as investigating early events. Studies tackling air pollution effects from different angles are needed to link together early events with the diseases of concern. As early events may be more susceptible to interventions aimed at reversing them or slowing their progression, we hope that our contribution to identify novel modifiable mechanisms induced by air particle exposures can help reach the goal recently set forth for environmental scientists by the newly appointed director of the National Institute of Environmental Health Sciences "to prevent or stop the progression of complex health problems" (Birnbaum 2009).

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Bisphenol, npcRNAs and Utero-Ovarian Feed-Back Control of Breast Cancer Chemosensitivity

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In the February 2009 issue of *Environmental Health Perspectives*, La Pensee et al. (2009) postulated that bisphenol A (BPA), at nanomolecular doses, confers chemoresistance in estrogen receptor (ER)-α–positive and –negative breast cancer cells. Certainly, drug resistance is well-known to be an important complication in a variety of cancer chemotherapy options. Several molecular mechanisms have been suggested to explain the onset of drug resistance. Determining the exact mechanism in a particular case is challenging both at the clinical and preclinical research levels because the genome-wide and proteomic approaches to mechanistic studies are still at a developing stage (Zhang and Liu 2007).

With regard to cisplatin, doxorubicin, and vinblastine cytotoxicity, there are proposed mechanisms of cytotoxicity in breast cancer cells and cell lines other than ER or receptor-mediated molecular signals. These mechanisms involve not only apoptosis pathways but also other regulatory, functional, and structural mechanisms of phenotypic expression in breast cancer models that could interfere with androgen receptor-mediated transcriptional activities (Aube et al. 2008). In addition, after the invention of microarray systems, more focus has been placed on the large number of human transcripts that have been described but do not code for proteins, such as nonprotein coding RNAs (Mallardo et al. 2008). These may include subfractions of small (microRNAs, small nucleolar RNAs) and long RNAs (antisense RNA, double-stranded RNA, and long RNA species) that function as regulators of other mRNAs at the transcriptional and post-transcriptional levels and control protein ubiquitination and degradation, with possible other roles yet to be elucidated. Various species of nonprotein-coding RNAs (npcRNAs) have been found to be differentially expressed in diverse types of cancer, including breast cancer subtypes (Mallardo et al. 2008). Because BPA is a highly reactive chemical, it would not be surprising if it interacts with some of these npc RNAs that could mediate ER response.

Recent reports from other laboratories have tended to support a role of npcRNAs in BPA-mediated mechanisms involved in breast cancer and a possible physiochemical interaction of BPA with estrogen and non-estrogen-mediated chemosensitivity-inducing pathway elements. For example, Hong et al. (2006) used expression microarray technology to predict hormone-responsive activities in response to estrogen and endocrine disruptors. According to these authors, the expression levels of only 555 genes (7.42%) among the 7,636 genes spotted on microarray chips were enhanced by > 2-fold after treatment with estradiol (E_2), suggesting that direct or rapid response to E_2 is widespread at the mRNA levels in these genes. Hong et al. (2006) observed that elevated expression levels of the genes (over 2-fold) were induced by BPA (8.26%) in the uterus of immature rats. Examples of

differentially expressed representative genes include calbindin-D9k (vitamin D-dependent calcium-binding protein), oxytocin, adipocyte complement related protein (30 kDa), lactate dehydrogenase A, and calcium-binding protein A6 (calcyclin). The mRNA levels of these genes were also increased in various phases of the menstrual cycle. This study in rats (Hong et al. 2006) supports the possibility of distinct effects of endogenous E_2 and environmental endocrine-disrupting chemicals in the uterus of women. Involvement of these gene transcripts, which are present in breast, uterine, and ovarian tissues, in the environment–endocrine interaction suggests the possibility of a utero-ovarian feedback control of breast cancer chemosensitivity effected by npcRNAs.

Sladek and Somponpun (2008) studied the effect of vasopressin (VP) on the reproductive cycles of humans; their results suggest the involvement of multiple types of ERs in the VP-mediated G-protein coupled response (Hong et al. 2006). VP, acting through fluid-electrolyte mechanisms, may have a role in the mechanism of breast cancer initiation and progression, which may be prone to regulatory impacts through npcRNAs involved in the replication of dysregulating pathways of the mammary epithelium.

These observations suggest that there may be a utero-ovarian feedback control mediated by ERs on the uterus that cross-talk with vasoneural pathways; the feedback control may mediate estrogen involved in chemoresponsive pathways of breast cancer. Furthermore, these pathways may be regulated by noncoding npcRNAs whose functions may be physiochemically modified by environmental toxicants such as BPA and other related chemials.

Since the forum titled "Bisphenol A: An Expert Panel Examination of the Relevance of Ecological, *in Vitro* and Laboratory Animal Studies for Assessing Risks to Human Health" in Chapel Hill, North Carolina, on 28–30 November 2006 (Keri et al. 2007), there has been no meeting convened to discuss environmental endocrine disruptors, particularly as it relates to BPA and related chemicals. I hope that a future review panel will assess the literature on both animals and humans and evaluate the role of BPA in carcinogenesis. Such an assessment should also include recommendations for future areas of research.

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Editor's note: In accordance with journal policy, LaPensee et al. were asked whether they wanted to respond to this letter, but they chose not to do so.

Errata

In the June Science Selection article "Prenatal Preview: Early Bisphenol A Exposure May Spawn Late-Life Reproductive Problems" [Environ Health Perspect 117:A256 (2009)], diethylstilbestrol is incorrectly identified as an antinausea drug. This drug was actually used to prevent miscarriage. *EHP* regrets the error.

In the "Discussion" (paragraph 14, p. 701) of the article by Guidotti et al. [Environ Health Perspect 115:695–701 (2007)], the first two sentences ("There appears to have been no identifiable public health impact from the elevation of lead in drinking water in Washington, DC, in 2003 and 2004. This may reflect effective measures to protect the residents, as 153 reported compliance with recommendations to filter their drinking water.") should have been replaced with the following sentence: "Measures to protect residents from exposure to lead in drinking water may have prevented more frequent elevations in blood lead." In addition, on page 695 in the right-hand column, line 4, the year 2002 should be given as 2000. The authors apologize for these errors.

In Figure 6C of Moors et al. [Environ Health Perspect 117:1131–1138 (2009)], the symbols for control and 1 μm STS were not correct. The corrected figure is presented here. *EHP* regrets the error.